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OSTEOARTHRITIS and CARTILAGE

Osteoarthritic synovial fluid and synovium supernatants up-regulate tumor necrosis factor receptors on human articular chondrocytes

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Summary

Objective: To determine whether the up-regulation of chondrocyte tumor necrosis factor receptor (TNF-R) expression in osteoarthritis (OA) is due to molecules released within the OA knee joint.

Design: Non-arthritic (NA) human articular chondrocytes were incubated with normal serum, OA synovial fluid, or supernatants from either cultured NA or OA synovium, and TNF-R expression measured by flow cytometry.

Results: OA synovial fluid, but not normal serum, significantly up-regulated the proportion of chondrocytes expressing p55 TNF-R as well as the number of p55 TNF-R/chondrocyte. Similarly, supernatants from OA, but not NA, synovia significantly up-regulated chondrocyte p55 TNF-R expression. Chondrocyte p75 TNF-R expression was also significantly increased by some of the OA supernatants but not others, and overall no significant increase was seen. OA synovium supernatants contained higher concentrations of interleukin-1β (IL-1β) and interleukin-6 (IL-6) than NA synovium supernatants and neutralizing antibodies to these cytokines either partially or totally abrogated the ability of the OA supernatants to increase chondrocyte p55 TNF-R expression. Finally, various concentrations of recombinant human (rh)IL-1β and rhIL-6 up-regulated chondrocyte p55 TNF-R expression.

Conclusion: These results suggest that IL-1 and IL-6 produced by OA synovium contribute to the progression of the disease by rendering chondrocytes more susceptible to stimulation by catabolic cytokines.

Key words: Osteoarthritis, TNF-receptors, Human articular chondrocytes, IL-1, IL-6.

Introduction

Osteoarthritis (OA) is characterized by focal loss of articular cartilage. Although the etiology of OA remains uncertain, it is likely that a number of separate disease processes converge on a 'final common pathway' culminating in joint damage [1, 2]. The exact sequence, interrelationship and cause of the changes leading to such damage, however, are not well documented. The integrity of normal cartilage is maintained by the delicate balance between cytokine-driven anabolic and catabolic processes. This system of homeostasis is further 'fine tuned' by regulators of matrix turnover such as tissue inhibitor of metalloproteases (TIMP) which control the activities of metalloproteases. In OA the net loss of proteoglycans and the destruction of the collagen network within the cartilage is thought to be caused by a shift in equilibrium toward catabolism. This theory is borne out by the fact that in OA cartilage there is an excess of protease compared with TIMP [3] and that OA synovium produces more catabolic cytokines, such as interleukin-1β (IL-1β) [4, 5] and tumor necrosis factor α (TNFα) [5] than non-arthritic (NA) synovium.

Previous work has shown that OA cartilage is more susceptible to the effects of both TNFα [6, 7] and IL-1β [8] than NA cartilage, and that OA chondrocytes express more of the receptors involved in signalling for TNFα (p55) [9] and IL-1 (type I) [10] than NA chondrocytes. The question thus arises as to what stimulates OA chondrocytes to express increased levels of these receptors. It is known that synovial inflammation and increased subchondral bone activity can predict OA disease progression [11, 12]. It might therefore be expected that factors elaborated by these compartments are those which stimulate OA chondrocytes to express increased numbers of catabolic cytokine receptors. The purpose of the current work was to determine if OA synovial fluid and/or supernatants from cultured OA synovium up-regulate p55 TNF

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receptor (TNF-R) expression on NA chondrocytes and if so, to identify the agent(s) responsible.

Materials and Methods

SERUM AND SYNOVIAL FLUID

Normal human serum was obtained from healthy volunteers at Bristol University and pooled before use. SF (mean volume 16 $\pm$ 11 ml, range 4–37) was aspirated from the knee joint of patients (N = 7; 3 males/4 females, mean age 65.1 $\pm$ 11.6 years, range 48–80 years) who fulfilled the American College of Rheumatology criteria for OA, when indicated for diagnostic or therapeutic reasons. The total white blood cell count of the SF was $6\times10^5 \pm 12.8\times10^5$ (range $<10^5$–35×10^5).

CARTILAGE

NA cartilage was obtained from the femoral heads of patients undergoing hemiarthroplasty following osteoporotic subcapital fracture of the femoral neck. In these patients (N = 29; 12 males/17 females, mean age 82 $\pm$ 7.3 years, range 60–94 years) there was no clinical evidence of arthritis. The cartilage was macroscopically normal.

CHONDROCYTE ISOLATION

Slices of cartilage, taken less than 6 h postoperatively, were removed under sterile conditions using a scalpel. The cartilage was finely chopped into cubes (2–3 mm^3) and the chondrocytes released by sequential enzymatic digestion as previously described [9]. The resulting digest was filtered (40 μm, Falcon) to remove any debris and the chondrocytes washed in HAMS F12 medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM HEPES and 10% foetal calf serum (FCS) (complete HAMS). The viability of the chondrocytes as assessed by trypan blue exclusion was 95.2 $\pm$ 3.2% (range 90–99). To allow TNF-R to return to pre-treatment levels [13, 14], chondrocytes were rested for 16 h (37°C, 5% CO_2) in flasks coated with 2% agarose (Type VII, Sigma) [9].

CULTURE OF CHONDROCYTES WITH SF

Following overnight (16 h) incubation the chondrocytes were harvested and washed by centrifugation in complete HAMS (1000 rpm, 7 min). Chondrocytes (5×10^5/ml) were further incubated with complete HAMS or complete HAMS supplemented with 50% SF. As a control, chondrocytes were also cultured in the presence of 50% normal serum. All incubations were carried out in agarose-coated 24-well plates (Falcon) (24 h, 37°C, 5% CO_2), after which time chondrocytes were removed and prepared for staining.

SYNOVIUM

NA synovium was obtained from the hip joint of patients (N = 2F, aged 74 and 82 years) undergoing hemiarthroplasty following osteoporotic subcapital fracture of the femoral neck and from one normal knee joint [1 (male), age 67 years] less than 48 h *post-mortem*. As a further control, tissue was obtained from within the medullary canal of one patient [1 (male), age 25 years] at the time of removal of an intramedullary nail inserted 18 months previously for treatment of tibial shaft fracture. Such tissue has previously been used as normal material where the fracture was soundly united and there was no evidence of osteolysis [4].

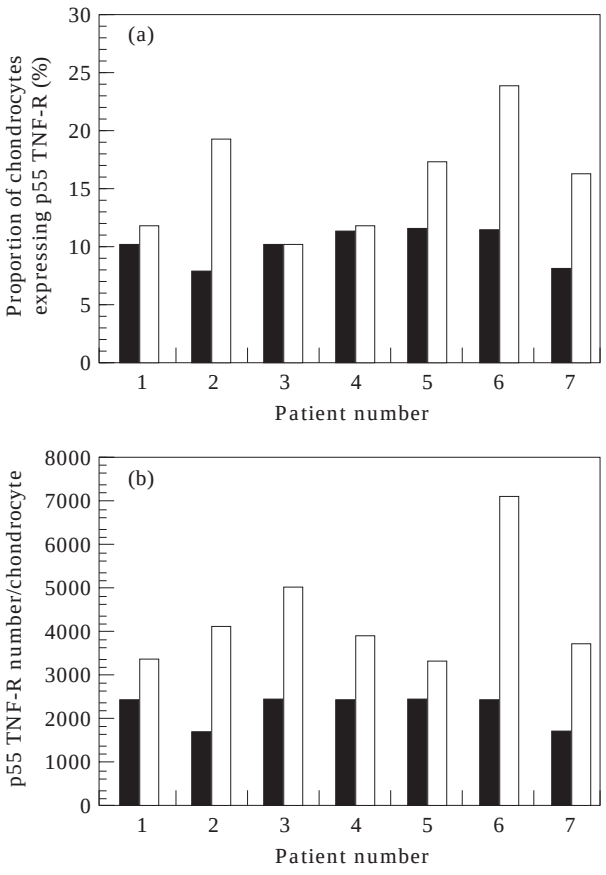

FIG. 1. Effect of normal human serum and OA synovial fluid on (a) the proportion of NA chondrocytes expressing p55 TNF-R and (b) the relative number of p55 TNF-R/chondrocyte. Chondrocytes were cultured in either normal human serum (■) or OA SF (□) for 24 h and stained for TNF-R.

Clinical evidence of arthritis was absent in all NA patients. Synovium was obtained from OA patients ($N = 6$, 4 males/2 females, mean age 68.8 ± 3.4, range 65–74 years) who fulfilled the American College of Rheumatology criteria, undergoing surgery for total knee replacement. One patient, undergoing bilateral total knee replacement, provided a specimen of synovium from each knee.

SYNOVIUM CULTURE

Synovium was aseptically removed from the femoral heads and knee joints using scissors, weighed and cut into small pieces (2–3 mm^3). The synovium was washed in phosphate-buffered saline (PBS) and cultured in RPMI supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 25 mM HEPES and 5% FCS

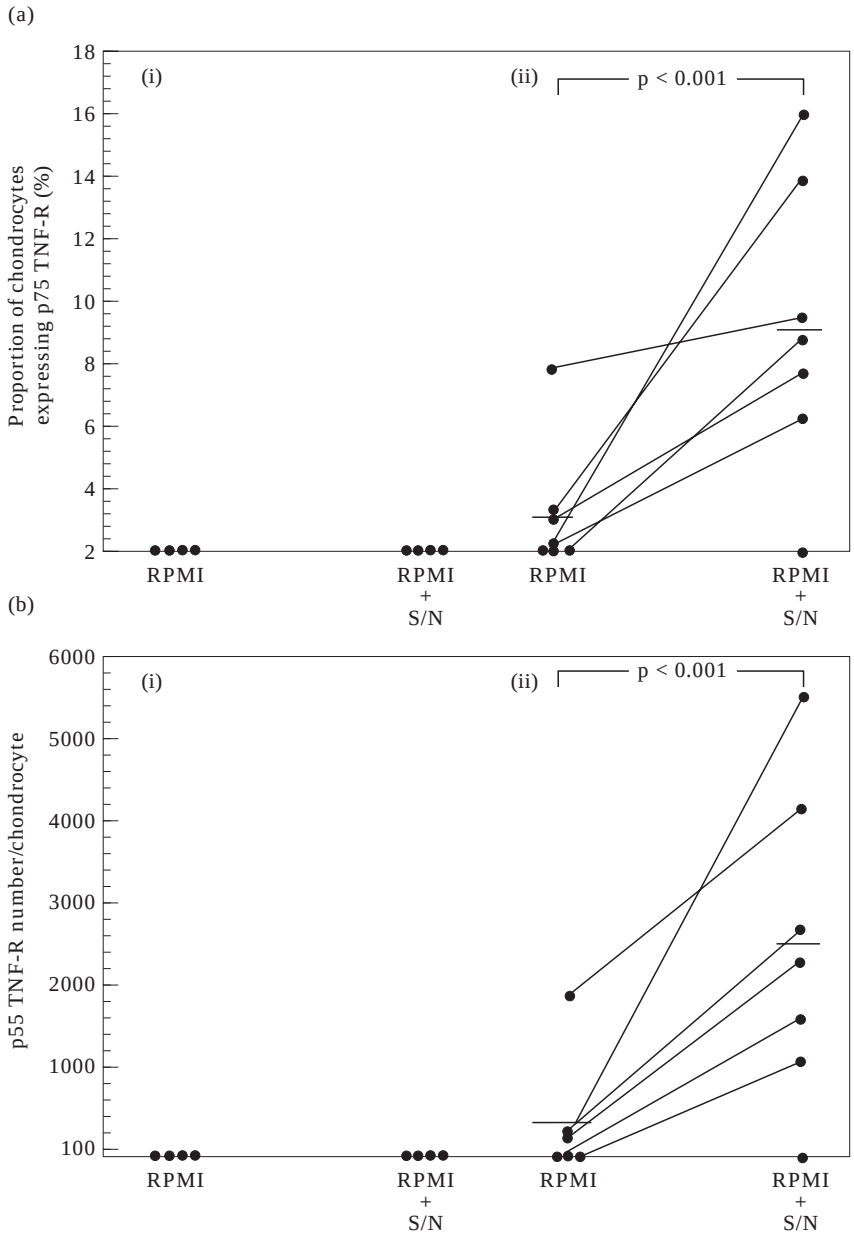

FIG. 2. Effect of synovium supernatants on (a) the proportion of NA chondrocytes expressing p55 TNF-R and (b) the relative number of p55 TNF-R/chondrocyte. Chondrocytes were cultured in (i) medium or medium containing 25% NA synovium supernatant (NA = 4) or (ii) medium or medium containing 25% OA synovium supernatant (OA = 7) for 24 h and stained for TNF-R expression. Each point represents the mean value of experiments performed on chondrocytes prepared from between two and five different cartilage donors. Each line joins the values obtained for chondrocytes cultured in the presence and absence of one synovium supernatant. The heavy bars indicate the mean value for each group.

(0.2 g of tissue/1 ml RPMI). After incubation for 24 h (37°C, 5% CO_2) the supernatant was aspirated from the tissue, filtered (40 μm, Falcon), centrifuged (4000 rpm, 10 min) to remove any fat and cellular debris and stored in aliquots at –70°C.

CULTURE OF CHONDROCYTES WITH SYNOVIUM SUPERNATANT

Chondrocytes were harvested, washed by centrifugation in RPMI supplemented with 5% FCS (1000 rpm, 7 min) and cultured in RPMI or RPMI

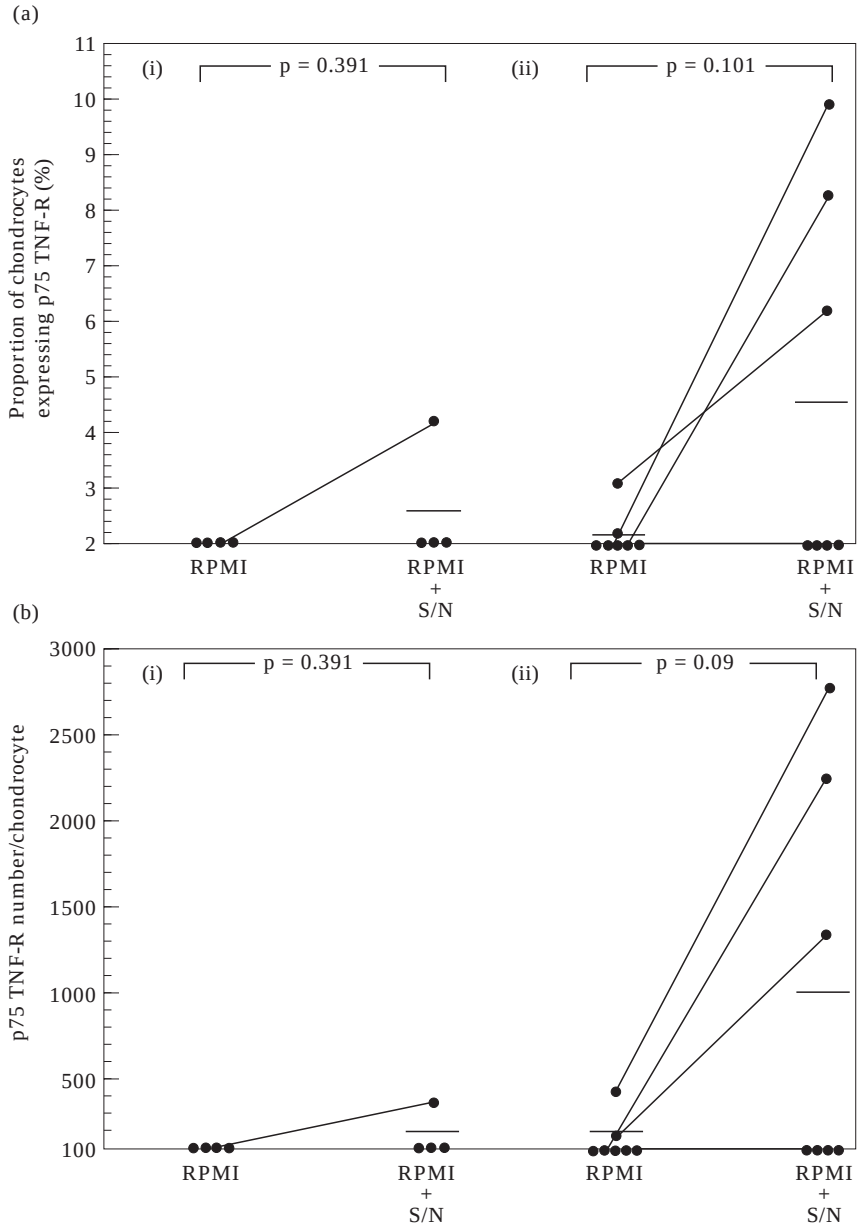

FIG. 3. Effect of synovium supernatants on (a) the proportion of NA chondrocytes expressing p75 TNF-R and (b) the relative number of p75 TNF-R/chondrocyte. Chondrocytes were cultured in (i) medium or medium containing 25% NA synovium supernatant (NA = 4) or (ii) medium or medium containing 25% OA synovium supernatant (OA = 7) for 24 h and stained for TNF-R expression. Each point represents the mean value of experiments performed on chondrocytes prepared from between two and five different cartilage donors. Each line joins the values obtained for chondrocytes cultured in the presence and absence of one synovium supernatant. The heavy bars indicate the mean value for each group.

supplemented with 25% synovium supernatant. After incubation (37°C, 5% CO_2, 24 h) cells were removed and prepared for staining. All incubations were carried out in agarose-coated 24-well plates (Falcon). Each synovium supernatant was tested on chondrocytes prepared from between two and five different cartilage donors.

MEASUREMENT OF IL-1β IN SYNOVIUM SUPERNATANTS

IL-1β was measured in supernatants by a commercially available enzyme-linked immunosorbent assay (ELISA) (Biotrak, Amersham). The kit was assessed for use with culture supernatants. A subset of supernatants was serially diluted in the assay to test linearity of the response and determine optimal dilution (neat–1/5). Linearity was obtained in the IL-1β assay. Spiking experiments were performed and the recovery of added IL-1β was 89.5% (range 80.7–98.1).

MEASUREMENT OF IL-6 IN SYNOVIUM SUPERNATANTS

IL-6 was measured as previously described [15] and the measurements were subjected to PROBIT analysis [16] due to non-linearity.

INCUBATION WITH NEUTRALIZING ANTIBODIES

To determine the possible identity of the up-regulatory factor(s), chondrocytes were cultured in the presence of 25% synovium supernatants containing either normal goat serum or goat anti-serum directed against either IL-1α and IL-1β (NIBSC, 1/250: 1/250) or IL-6 (NIBSC, 1/750). In three experiments sufficient IL-6 (R&D Systems, 10 ng/ml) to neutralize the effects of the antibody was added to chondrocytes cultured with 25% synovium supernatant and anti-IL-6. Similarly in three experiments sufficient IL-1β (NIBSC, 1 ng/ml) to neutralize the effects of the antibody was added to chondrocytes cultured with 25% synovium supernatant and anti-IL-1α and β. After incubation (37°C, 5% CO_2, 24 h) chondrocytes were removed and prepared for staining.

In some experiments chondrocytes were incubated with RPMI alone or in the presence of varying concentrations of either rhIL-1β or rhIL-6. After incubation (37°C, 5% CO_2, 24 h) chondrocytes were removed and prepared for staining.

CELL PREPARATION AND STAINING

The chondrocytes were washed twice in 2 M sodium chloride/20 mM HEPES buffer (pH 7.4) and

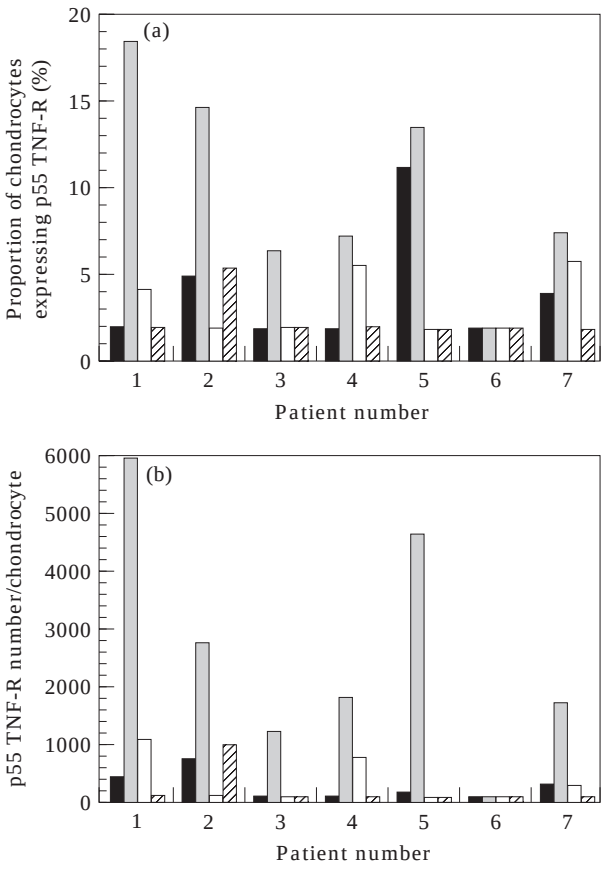

FIG. 4. Ability of neutralizing antibodies to IL-1α and IL-1β or anti-IL-6 to inhibit the up-regulatory effect of synovium supernatants on (a) the proportion of chondrocytes expressing p55 TNF-R and (b) the relative number of p55 TNF-R/chondrocyte. NA chondrocytes were incubated in medium alone (■) or medium containing 25% OA synovium supernatant (▨) or medium containing 25% synovium supernatant and either mixtures of neutralizing antibodies to IL-1α and IL-β (□) or anti-IL-6 (▨). The chondrocytes were stained for TNF-R after 24 h.

once in 2 M sodium chloride/20 mM sodium acetate (pH 4) to remove any bound ligand [9]. The viability of the chondrocytes was assessed again and was 75 ± 14%. U937 cells, a monocytic cell line known to express TNF-R [17], were used as a positive control; Raji cells, a B-cell line reported not to express TNF-R [18], was used to determine nonspecific binding [19]. The chondrocytes and control cells were stained for p55 and p75 TNF-R expression using non-cross-reacting antibodies [19] and analysed by flow cytometry as previously described [9]. Briefly, chondrocytes and control cells (5×10^5/25 µl) were incubated (45 min, 4°C) with mouse monoclonal antibodies (kind gift of Dr M. Brockhaus, Hoffman La Roche, Basel) to either the p55 receptor (htr9), the p75 receptor (utr1), or with IgG_1 isotype control (Becton Dickinson). All

Table I
Effect of normal goat serum on upregulation of p55 TNF-R expression by chondrocytes cultured in the presence of an OA synovium supernatant (s/n)

Culture conditions	Proportion of chondrocytes expressing p55 TNF-R (%)	p55 TNF-R number/ chondrocyte
RPMI alone	5.4 ± 2.0	614 ± 405
25% synovium s/n	7.7 ± 0.3	1933 ± 289
25% synovium s/n + normal goat serum	8.9 ± 1.9	1936 ± 269

$N = 3$.

cells were washed and incubated (15 min, 4°C) with goat anti-mouse IgG conjugated to fluorescein-isothiocyanate (Dako). The cells were then washed and resuspended in 250 µl PBS/0.25% formaldehyde before analysis (FACScan, Becton Dickinson). Comparison was made between cells stained with specific monoclonal antibodies and cells from the same population stained with the appropriate isotype control. Results were calculated by determining the shift in fluorescence of the test population compared with control and expressed as the proportion of positive cells in the population analysed (5000 cells). The reproducibility of the calculation of the percentage expression, the co-efficient of variance (CV), was 8.9 ± 0.8% and the limit of detection 2%.

To estimate the number of receptors/cell, beads exhibiting various numbers of mouse IgG molecules (Dako, Qifi-kit) were incubated, at the same time as cells, with fluorescein-labelled goat anti-mouse IgG. A standard curve of mean fluorescence intensity against antigen density was constructed using the beads. The net fluorescence intensity of the chondrocytes run at the same setting was measured. The relative receptor number on the chondrocytes (which is directly proportional to the antigen density) was calculated from the standard curve. The reproducibility of the estimation of receptor number/chondrocyte (CV) was 8.7 ± 1.0% and the limit of detection 100 TNF-R/chondrocyte.

STATISTICAL ANALYSIS

The Student's paired *t*-test was used to analyse the differences between stimulated and unstimulated NA chondrocyte populations. Differences between OA and NA synovium supernatants and differences between supernatants tested on different chondrocyte populations were analysed by two-way analysis of variance (ANOVA).

Results

EFFECTS OF OA SF ON NA CHONDROCYTE TNF-R EXPRESSION

NA chondrocytes were incubated with either a pool of normal serum or SF from OA patients and the proportion of chondrocytes expressing p55 TNF-R and the numbers of p55 TNF-R/chondrocyte measured. The results for seven different SF are illustrated in Fig. 1(a) and (b). p55 TNF-R expression was increased by all the OA SFs as compared with normal serum. Both the proportion of chondrocytes expressing p55 TNF-R and the number of p55 TNF-R/chondrocyte were significantly increased by OA SF ($P < 0.014$ and $P < 0.005$, respectively) as compared with chondrocytes incubated with normal serum. It should be noted that the chondrocyte TNF-R expression was unaffected by normal serum and therefore represents the basal level. The proportion of chondrocytes expressing p55 TNF-R after culture in medium was 4.5 ± 6.1% and in normal serum

Table II
Effect of IL-6 on the neutralizing ability of anti-IL-6 on chondrocytes cultured in the presence of an OA synovium supernatant (s/n)

Culture conditions	Proportion of chondrocytes expressing p55 TNF-R (%)	p55 TNF-R number/ chondrocyte
RPMI alone	2.0 ± 2.8	164 ± 232
25% synovium s/n	7.6 ± 0.5	2394 ± 361
25% synovium s/n + anti-IL-6	2.9 ± 4.1	71 ± 120
25% synovium s/n + anti-IL-6 + IL-6	8.3 ± 0.8	2915 ± 66

$N = 3$.

Table III
Effect of IL-1β on the neutralizing ability of anti-IL-1α/β on chondrocytes cultured in the presence of an OA synovium supernatant (s/n)

Culture conditions	Proportion of chondrocytes expressing p55 TNF-R (%)	p55 TNF-R number/ chondrocyte
RPMI alone	2	100
25% synovium s/n	9.0 ± 1.2	2849 ± 787
25% synovium s/n + anti-IL-1	2	100
25% synovium s/n + anti-IL-1 + IL-1	9.2 ± 0.9	3223 ± 325

$N = 3$.

5.8 ± 5.5% ($N = 5$, $P = 0.23$). The number of p55 TNF-R/chondrocyte in medium was 830 ± 1166 and in normal serum 1491 ± 1362 ($N = 5$, $P = 0.42$). The proportion of chondrocytes expressing p75 TNF-R and the number of p75 TNF-R/chondrocyte was below the detection limit before and after incubation with OA SF.

EFFECT OF SYNOVIUM SUPERNATANT ON NA CHONDROCYTE TNF-R EXPRESSION

Experiments were performed to determine whether the TNF-R up-regulating activity present in OA SF could be due to release of products by cells in diseased synovium. NA chondrocytes were incubated with medium alone or with supernatants from cultured NA synovium and the effect on TNF-R expression measured. As can be seen from Fig. 2 (a)i, (b)i, NA synovium supernatants had no significant effect on either the proportion of chondrocytes expressing p55 TNF-R or on the number of p55 TNF-R/chondrocyte as compared with medium alone. Similar experiments were performed with medium and supernatants from OA synovia. Supernatants from OA synovia significantly increased the proportion of NA chondrocytes expressing the p55 TNF-R ($P < 0.001$) [Fig. 2(a)ii] and up-regulated the number of p55 TNF-R/chondrocyte ($P < 0.001$) [Fig. 2(b)ii] as compared with chondrocytes cultured in medium alone. It can also be seen from Fig. 2 that p55 TNF-R expression varied on chondrocytes cultured in medium alone. However, the effect of OA synovium supernatants was consistent. For example, the proportion of chondrocytes expressing p55 TNF-R isolated from three different individuals and incubated with medium was <2, <2 and 4.9%, respectively, while after culture with a synovium supernatant from one patient the proportion was 14.8, 17.1 and 13.2%, respectively. Similarly the numbers of p55 TNF-R on the three chondrocyte preparations incubated in medium was <100, <100 and 724 and with the synovium supernatant 2695, 1914, 2754, respectively.

It can be seen from Fig. 3 that one out of four NA synovium supernatants and three out of seven OA synovium supernatants significantly increased both the proportion of chondrocytes expressing p75 TNF-R and the number of p75 TNF-R /chondrocyte. However, overall both NA and OA synovium supernatants had no significant effect on either the proportion of chondrocytes expressing p75 TNF-R or on the number of p75 TNF-R/chondrocyte as compared with medium alone.

EFFECT OF NEUTRALIZING ANTIBODIES ON THE UP-REGULATION OF TNF-R EXPRESSION BY SYNOVIUM SUPERNATANTS

Both IL-1β [20] and IL-6 [21] are known to up-regulate TNF-R on various cell types. These observations led us to consider that these cytokines may contribute to the effects of OA synovium supernatants on chondrocyte TNF-R expression. As a first step towards examining this possibility, IL-1β and IL-6 concentrations were measured in the supernatants. The concentration of IL-1β in the four NA synovium supernatants was 7.7 ± 7 pg/ml (range ≤3–17) and in the seven OA supernatants was 344 ± 242 pg/ml (range 48–691). The concentration of IL-6 in NA supernatants was 0.8 ± 0.8 ng/ml (range ≤0.1–1.6) and in the OA supernatants was 6.4 ± 4.5 ng/ml (range 0.12–15.2). Next, neutralizing antibodies to IL-1 or IL-6 were added to supernatants at the time of incubation with chondrocytes and the effects on TNF-R measured. The results are recorded in Fig. 4(a and b). It is evident that both mixtures of anti-IL-1α and β, and anti-IL-6 significantly ($P < 0.001$ and $P < 0.001$, respectively) decreased the proportion of chondrocytes expressing the p55 TNF-R as compared with chondrocytes incubated in the presence of OA synovium supernatant alone [Fig. 4(a)]. Similarly, both mixtures of anti-IL-1α and β, and anti-IL-6 significantly ($P < 0.001$ and

$P < 0.001$, respectively) decreased the number of p55 TNF-R/chondrocyte [Fig. 4(b)].

In concurrent experiments, chondrocytes were incubated with supernatant (25%) or supernatant containing normal goat serum (1/250 dilution) and TNF-R expression measured. From Table I it can be seen that normal goat serum failed to inhibit the effects of synovium supernatant on either the proportion of cells expressing p55 TNF-R or the number of p55 TNF-R/chondrocyte.

The specificity of the anti-IL-6 and anti-IL-1α and β serum was also tested by attempting to reverse the effects of the antibody by addition of exogenous rhIL-6 or rhIL-1*β*. Chondrocytes were cultured in medium alone, medium containing a synovium supernatant (25%), medium containing a synovium supernatant (25%) and either anti-IL-6 or anti-IL-6 and IL-6 (10 ng/ml), and TNF-R expression measured. Table II shows that the addition of rhIL-6 restored the proportion of cells expressing p55 TNF-R and the number of p55 TNF-R/chondrocyte to the levels seen on chondrocytes incubated in the presence of 25% synovium supernatant alone. Analogous experiments carried out with anti-IL-1α/β and rhIL-1β gave a similar result (Table III).

Finally, the effects of rhIL-1β and rhIL-6 on chondrocyte p55 TNF-R expression were measured. NA chondrocytes were incubated with increasing concentrations of either rhIL-1β or rhIL-6. Fig. 5 shows that IL-1β increased p55 TNF-R expression at concentrations of 1 and 10 pg/ml but not at 100 pg/ml, while IL-6 increased p55 TNF-R expression at concentrations from 100 pg/ml to 10 ng/ml but not at 20 ng/ml or above. It should be noted that no significant difference in cell viability was found between chondrocytes cultured in the presence of low or high concentrations of either cytokine.

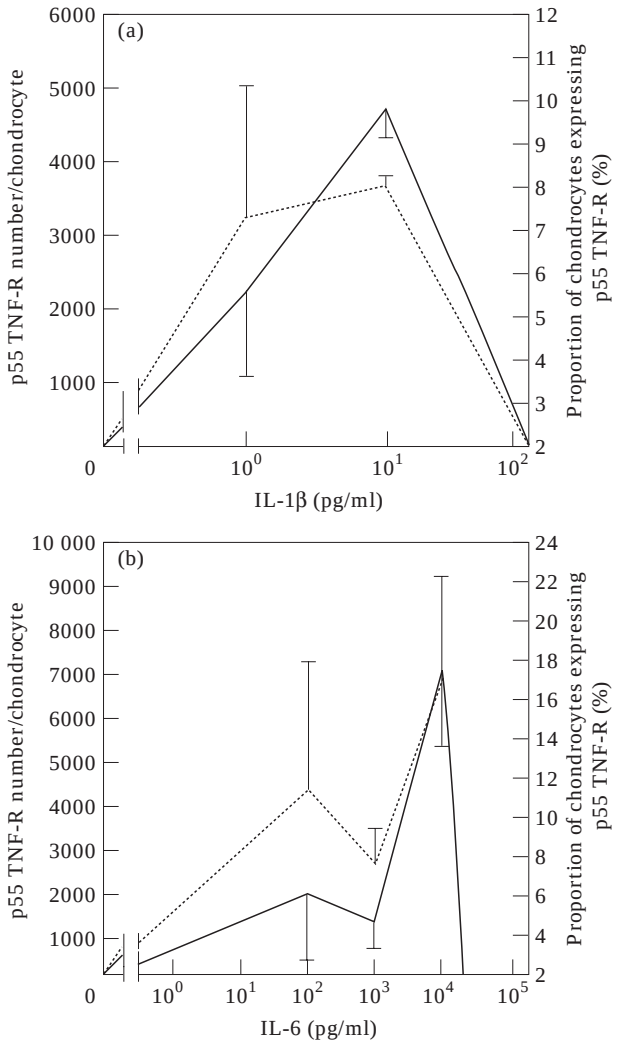

FIG. 5. Effect of IL-1β (a) and IL-6 (b) on chondrocyte p55 TNF-R expression. The proportion of chondrocytes expressing p55 TNF-R is represented by (.....) and p55 TNF-R number/chondrocyte by (——). The means ± S.E.M. of three experiments performed on chondrocytes from different NA cartilage donors are shown.

Discussion

These results show that OA SF has the capacity to up-regulate p55 TNF-R expression on NA human articular chondrocytes. Both the proportion of chondrocytes expressing p55 TNF-R and the number of p55 TNF-R/chondrocyte were increased. It may be argued that a two- to threefold increase in p55 TNF-R expression is unlikely to have an affect on cartilage degradation. However, recent work in our laboratory showed that the susceptibility of cartilage explants to TNFα, as judged by glycosaminoglycan (GAG) loss, is directly related to chondrocyte p55, but not p75, TNF-R expression [6]. Moreover, a twofold increase in chondrocyte p55 TNF-R expression, on average, resulted in a two- to threefold increase in GAG release. Taken together these results suggests that the increase in chondrocyte p55 TNF-R reported here is biologically relevant.

Evidence that p55 TNF-R enhancing factors are produced locally in OA joints comes from the finding that supernatants from OA synovia significantly enhanced the proportion of chondrocytes expressing p55 TNF-R as well as the number of p55 TNF-R/chondrocyte. By contrast, supernatants from NA synovium had no effect on chondrocyte TNF-R expression. Synovial cells can produce a variety of pro- and anti-inflammatory cytokines and the concentrations of some are increased in disease [4, 5, 22–24]. It would appear

likely therefore that one or more of the latter are responsible for the effects seen.

What is the identity of the p55 TNF-R enhancing agent? The results point towards IL-1 and IL-6. This contention is supported by a number of observations. First, both cytokines were present in the OA synovium supernatants at higher concentrations than in the NA supernatants. Second, neutralization experiments demonstrated that the up-regulating effect of OA synovium supernatant on p55 TNF-R expression were inhibited by goat antisera to IL-1α/β and IL-6 but not normal goat serum. The specificity of the anti-sera was also confirmed by the fact that their corresponding recombinant cytokines reversed the inhibitory effects of the anti-sera. Third, chondrocyte TNF-R expression was increased by low concentrations of rhIL-1β and rhIL-6. By contrast high concentrations of rhIL-1β, comparable with those detected in some OA synovium supernatants, were without effect. It could be argued, therefore, that the results are contradictory. However, the supernatants were diluted for culture with chondrocytes thus bringing the concentration of IL-1β in most supernatants into the range at which rhIL-1β enhanced chondrocyte TNF-R expression. Moreover, inhibitory molecules produced by synovium, such as soluble IL-1R [25] and IL-1R antagonist [26], should both be present in the supernatants and would thereby reduce the biological activity of the IL-1β present. Finally, as already discussed, IL-1β may act together with other molecules such as IL-1α and IL-6 to enhance chondrocyte TNF-R expression.

Expression of p55 TNF-R and p75 TNF-R on a number of cell types is independently regulated [27, 28]. The results suggest that the same is true of chondrocyte TNF-Rs. OA SF enhanced chondrocyte p55 but not p75 TNF-R expression. Additionally, most synovium supernatants up-regulated p55 TNF-Rs whereas only some up-regulated p75 TNF-Rs.

Experimental models of OA have implicated both TNFα and its receptors as well as IL-6 in the disease process. For example, a generalized intensification of chondrocytic TNF-R staining with reduced metachromasia indicative of cartilage loss was noted in canine OA induced by cruciate ligament section [29]. In the same model, others reported elevated levels of IL-6 and TNFα in SF from the operated as compared with the sham operated joint. Moreover, SF TNFα concentrations correlated with proteoglycan loss from cartilage [30]. It is also accepted that IL-1 and TNFα could be involved in the OA process by virtue of their capacity to stimulate chondrocytes to degrade cartilage matrix and/or inhibit matrix synthesis. However, less attention has been paid to the possibility that the susceptibility of chondrocytes to catabolic stimuli affects the osteoarthritic process. This work shows that IL-1 and IL-6 produced by OA synovium increase p55 TNF-R expression which is known to result in increased susceptibility to TNFα-induced cartilage degradation [6]. These results thus advance the hypothesis that catabolic cytokines and their receptors have a role in the progression of OA.

Acknowledgments

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